Seminars in Cell & Developmental Biology 35 (2014) 126–135

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Chemoaffinity in topographic mapping revisited – Is it more about fiber–fiber than fiber–target interactions?

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ARTICLE INFO

Article history:
Available online 30 July 2014

Keywords:
Topographic map
Retinotectal
Axon guidance
Fiber–fiber interaction
Growth cone
Ephrin/Eph

ABSTRACT

Axonal projections between two populations of neurons, which preserve neighborhood relationships, are called topographic. They are ubiquitous in the brain. The development of the retinotectal projection, mapping the retinal output onto the roof of the midbrain, has been studied for decades as a model system. The rigid precision of normal retinotopic mapping has prompted the chemoaffinity hypothesis, positing axonal targeting to be based on fixed biochemical affinities between fibers and targets. In addition, however, abundant evidence has been gathered mainly in the 1970s and 80s that the mapping can adjust to variegated targets with stunning flexibility demonstrating the extraordinary robustness of the guidance process. The identification of ephrins and Eph-receptors as the underlying molecular cues has mostly been interpreted as supporting the fiber–target chemoaffinity hypothesis, while the evidence on mapping robustness has largely been neglected. By having a fresh look on the old data, we expound that they indicate, in addition to fiber–target chemoaffinity, the existence of a second autonomous guidance influence, which we call fiber–fiber chemoaffinity. Classical *in vitro* observations suggest both influences be composed of opposing monofunctional guidance activities. Based on the molecular evidence, we propose that those might be ephrin/Eph forward and reverse signaling, not only in fiber–target but also in fiber–fiber interactions. In fact, computational models based on this assumption can reconcile the seemingly conflicting findings on rigid and flexible topographic mapping. Supporting the suggested parsimonious and powerful mechanism, they contribute to an understanding of the evolutionary success of robust topographic mass wiring of axons.

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Abbreviations: a/p, anterior/posterior; FF, fiber–fiber; FT, fiber–target; fwd, forward; RGC, retinal ganglion cell; rev, reverse; SC, superior colliculus; t/n, temporal/nasal.

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http://dx.doi.org/10.1016/j.semcdb.2014.07.010
1084-9521/

1. Introduction

The connectionist view of neural function holds that the discriminating powers of perception, cognition, and motor control are embodied in the connectional architecture of the brain. The staggeringly complex connectome can roughly be classified into local and long-range wirings. There are two fundamental types of long-range axonal connections: non-topographic and topographic projections (Fig. 1A). In non-topographic projections, neuronal specification determines axonal destination, which is why they might also aptly be dubbed "typographic" projections. Their investigation has gained momentum only after the elucidation of the molecular organization of the prototypic olfactory system [1–3], because neuronal specificity is not similarly obvious in other non-topographic systems. Topographic projections, in contrast, are characterized by the preservation of spatial neighborhood relationships upon mapping, and examples are abundant. The topographic organization of the visual, auditory, somatosensory and motor systems have gained textbook prominence, but also deep inside the brain neighborhood preserving projections constitute a prevailing connectional motif [4], possibly because of its optimal wiring economy [5].

The developmental self-organization of the wiring first brings about the impressive computational capabilities of the brain and prearranges the tokens of the world eventually occurring to cognition. The elucidation of the pattern forming mechanisms of axon guidance has therefore intrigued neuroscientist for decades.

The systematic investigation of the mechanisms generating topographic projections originates in the early 1940s from the seminal work of Roger W. Sperry. He established the retinotectal projection, *i.e.*, the axonal connection between the retinal ganglion cells (RGCs) of the eye and the dorsal midbrain (tectum) in vertebrates, as an experimentally amenable model, hoped to be a more general paradigm. The retinotectal projection faithfully maps the retinal output onto the next level of the visual pathway. The global orientation of the retinotopic map (Fig. 1B) results in the projection of the temporal/nasal (t/n) and dorsal/ventral axes of the retina onto the anterior/posterior (a/p) and lateral/medial axes of the tectum, respectively. We will focus on the projection along the a/p axis, because dorsoventral mapping is thought to be independent and is less well understood. The establishment of the retinotopic map has been studied in teleost fishes, amphibians, birds and mammals (mammalian homolog: retinocollicular projection) [6–11]. In the chick, as an example, the axons of about 2.5 million RGCs per retina start to invade the tectum at its anteroventral pole at embryonic day 6 (E6) and spread out over the superficial fiber layer in posterodorsal direction until E13 to deploy the myriads of axonal terminals so that their arrangement ultimately reflects the neighborhood relationships of their retinal origins. When arrived near the topographically appropriate target, the axons dive down into the upper tectal layers and start to grow terminal arborizations [12]. Throughout this article, we will use the term "(axonal) terminal" collectively, to specify growth cones during development as well as maturing terminal arborizations. Interactions among these terminals and between the terminals and the target will be called "fiber–fiber" (FF) and "fiber–target" (FT) interactions, respectively, following a somewhat imprecise but common terminology. The mode of map formation differs somewhat between mammals and other vertebrates. In rats, the growth cones of RGC primary axons appear to be less responsive to a/p guidance cues, as most of them grow straight to the posterior end of the superior colliculus (SC). The correct termination zone is subsequently established by interstitial branching and elimination of the overshoot of the primary axon [13]. In lower vertebrates, in contrast, the growth cones of primary axons immediately target their destination [14]. Despite some overshoot [15], growth cones of chick RGC axons are exceedingly sensitive to the topographic guidance cues and the extent of overshooting is much more limited than in mammals [16]. It probably merely mirrors the general imprecision of primary targeting.

2. Organism level evidence – mapping rigidity and flexibility reconciled by fiber–fiber interactions

2.1. Mapping rigidity

The first principles of retinotopic map formation were gleaned from regeneration studies in anamniotes. In iconic experiments [17], Sperry transected the optic nerve in adult newts and rotated the eyeball by 180°. The visuomotor behavior of the recovered animal indicated an inversion and reversal of the visual field of the operated eye, suggesting the regenerated axons had precisely regrown to their original positions. This was later confirmed by histological fiber stainings in mature goldfish [18]. After nerve transection and deletion of any part of the retina, the remaining axons after about 1 month had rigidly homed in to their original target sites, bypassing the targets of the deleted retinal part. This led Sperry, drawing on ideas of Ramón y Cajal, to propose the chemoaffinity hypothesis [19]. It posits that targeting specificity of neurites be essentially based on selective biochemical affinities. This seminal notion founded the concept of genetic hard-wiring of the networks underlying major *a priori* capabilities of the brain. For topographic projections, Sperry suggested such chemical wiring cues might be implemented by complementary concentration gradients of at least one substance per axis on the connected fields, providing positional and directional information to the growing axons. These selective cytochemical affinities were conceived as relating to FT-interactions, although the chemoaffinity hypothesis does not logically require this constraint.

2.2. Mapping flexibility

The concept of rigid mapping was challenged immediately. In mature goldfish, after substantially longer regeneration times (≥3 month), a disconnected temporal half-retina (Fig. 1C) resulting from nerve transection and nasal ablation regenerated an expanded, proper projection covering the whole tectum ("expansion experiments"; [20]). Similar results were also obtained in Xenopus development. Double temporal (TT) or double nasal (NN) retinae microsurgically assembled in the larvae, instead of forming doubly occupied maps on their respective tectal halves, matured into expanded projections of each half-retina on the whole target ("compound eye experiments"; [21]). Conversely, tectal ablations (anterior or posterior half) in the goldfish (Fig. 1C), irrespective of concomitant nerve transection, yielded compressed maps of the full retina on the remaining half-target ("compression experiments"; [22–24]). Notably, without nerve transection, this implies mobilization of maturely connected axons of the remaining half-tectum. Thus, obviously, topographic mapping can flexibly adjust to the target.

2.3. Attempts to explain mapping flexibility

This flexibility reminds of activity-dependent plasticity. However, neither expansion in the goldfish [25], nor normal topographic mapping in lower vertebrates [26] or mammals [27] depends on impulse activity, but are genetically hard-wired. Early spontaneous activities, like retinal waves [28], seem to be important only after map establishment for compaction and elaboration of the axonal terminal arbors.

Sperry's hypothesis could easily be reconciled with flexible mapping, if the chemoaffinity labels had been altered by the microsurgical interventions. Controls involving secondary regenerations,

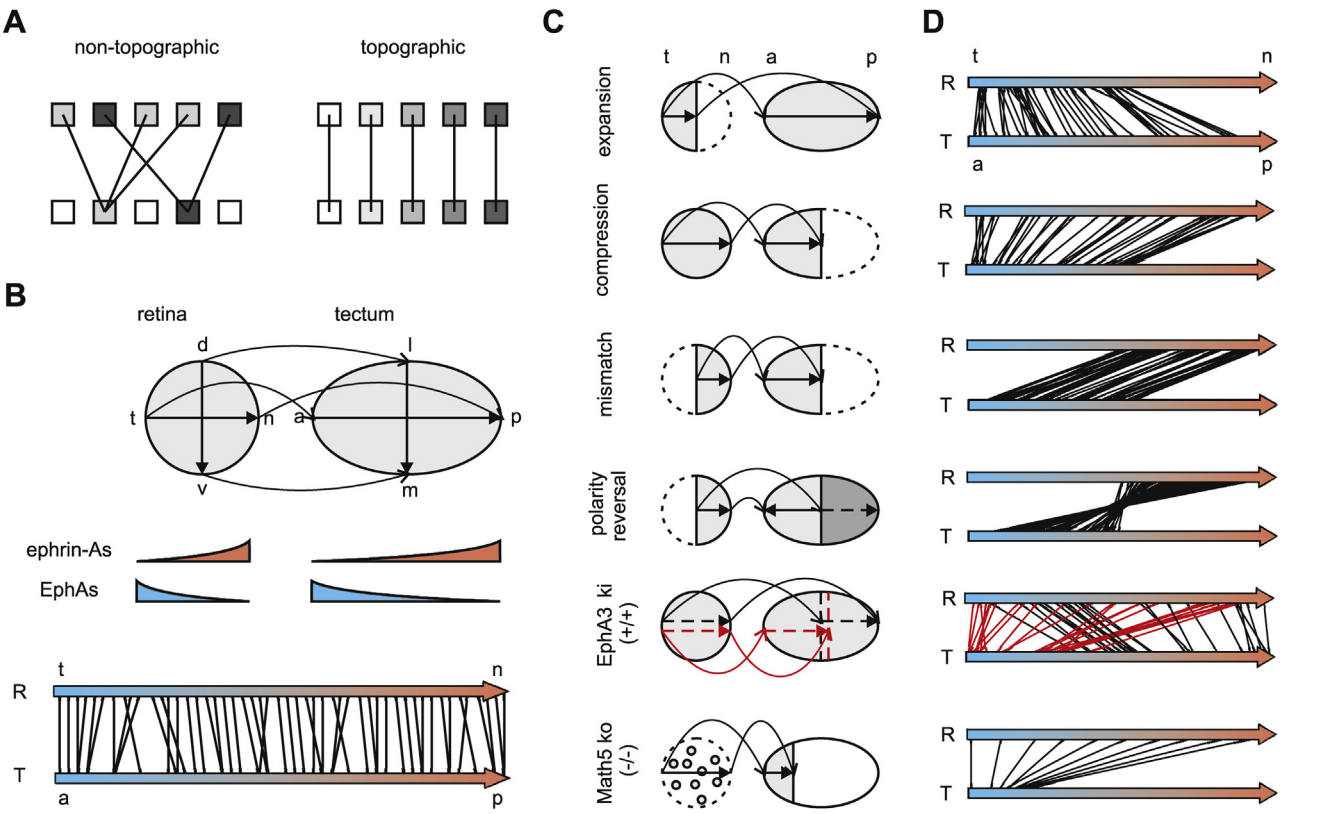

Fig. 1. Rigidity and flexibility of topographic mapping. (A) Non-topographic projections map cell specificity, topographic projections map spatial neighborhood. (B) Axial orientation of the retinotopic map (upper row), summed expression gradients of ephrin-As (red) and EphAs (blue) on retina and tectum displaying counter-distributions on both fields (middle), simulation of a normal retinotectal projection along the anterior/posterior axis, R: retina, T: tectum (lower row). Computational simulations were preformed employing the model and parameters given in [60] unless stated otherwise. Fifty terminals were randomly drawn from larger simulations and lines connecting their respective retinal origins and tectal targets are depicted here and in all parts of D. (C) Selected findings on mapping flexibility from microsurgical and genetic experiments. (D) Simplified simulation results (*cf.* A) gained with the model and parameters described in [60], which reproduce the experimental results symbolized in C. As a proof of principle, the Math5 knock-out phenotype was reproduced simulating the mapping of 10 axons randomly chosen from the whole retinal t/n extend and all are depicted here (parameters: $\gamma = 0.0125$, target field size 200×8, $C = 1$).

however, conclusively demonstrated that neither labels on the half-retinae [29,30] nor on the half-tectum [31] were permanently altered after successful flexible mapping. When ephrin-As had been identified as the major topographic cues, it could also be excluded that the terminals induced appropriate temporary markers only while present. In fishes, compared to normally innervated tecta, neither RNA [32,33] nor protein levels of ephrin-As [34] in radial glia cells, which commonly bear the guidance cues, significantly depended on the innervation state.

As markers are stable, while axons can flexibly map, chemoaffinity might not be absolute but relative. This idea was lucidly discussed already in 1975 [35] and has remained influential despite being hardly compatible with the existing evidence. It suggests a graded target label and all terminals seek one end of this gradient with topographically differential affinity. Then, topographically non-selective competition among the terminals could flexibly shift them, yielding topographic order. This would imply all terminals initially targeting the same preferred end and only subsequently being pushed to their proper targets. In fact, however, axons from TT retinae initially map to the anterior tectum before expanding posteriorly, whereas axons from NN retinae first project to the posterior tectum before expanding anteriorly [36]. Moreover, even the terminals of solitary zebrafish RGCs produced by single-cell transplantation into mutants lacking RGCs map to their topographically appropriate sites without any competition [33]. Thus, the combination of relative chemoaffinity and non-selective competition is insufficient to explain the experimental observations. The target by itself apparently carries sufficient topographic information to target the axons to their destinations.

What then mobilizes the terminals in the flexibility experiments without disrupting their topographic order? Compression without nerve transection suggests the regenerating homeless axons to mobilize the resident terminals. Albeit suggestive of simple competition, the interaction among the terminals, in fact, does not seem to be non-selective. When in goldfish or amphibians pieces of the tectum are translocated [37] or rotated [38,39], regenerating axons follow large grafts, but ignore small ones ("tectal graft experiments"). While the "follow" results corroborate the existence of positional and directional cues on the target, the "ignore" results clearly indicate that the surrounding terminals carry topographic information also, which they impose onto the terminals in the graft region, eventually superseding the altered target cues. Instead of non-selective competition, this suggests topographically selective FF-interactions. This type of interplay might be called FF-chemoaffinity. Conceivably, FF-chemoaffinity could be based on similar markers as FT-chemoaffinity, but with the cues being carried by the population of motile fiber terminals instead of being tethered to the target. Assuming initial FT- and subsequent FF-interactions would reconcile the chemoaffinity hypothesis with mapping rigidity and flexibility (see Section 5).

FF-chemoaffinity would predict fiber self-sorting capabilities. This is, in fact, supported by the "ignore" results after tectal grafting and two other sets of regeneration experiments. Simultaneous deletion of the temporal retina and the posterior tectum in the

goldfish (Fig. 1C; "mismatch experiments"; [40]) generated a smooth map of the residual nasal retina on the anterior tectum despite non-matching FT-chemoaffinity labels. When in a similar experiment the posterior tectum remained in place and occupied by its original innervation (Fig. 1C), a second re-growing nasal population again mapped onto the anterior half-tectum, now, however, with reversed polarity ("polarity reversal experiment"; [41]), *i.e.*, even against the instructions of the target markers. Mapping in these cases seems to be achieved by fiber self-sorting through FF-chemoaffinity.

Remarkably, even severed fibers appear to induce FF-chemoaffinity interactions. In older goldfish, distal axon stumps after nerve transection degenerate very slowly [42]. Hence, after short regeneration times, re-growing axons seem to be primarily guided by the remnants of the previous innervation and flexibly relocate only upon degeneration of the latter. Thus, curiously, Attardi's and Sperry's classical results, prompting the FT-chemoaffinity hypothesis, were actually most likely due to FF-chemoaffinity.

In summary, with regard to the mechanism of topographic guidance, the findings of the *in vivo* micromanipulation experiments indicate the existence of two topographically differential influences, FT- and FF-chemoaffinity, acting autonomously on the migrating terminals.

3. System level evidence from *in vitro* experiments supports both, fiber–target and fiber–fiber chemoaffinity

Historically, Friedrich Bonhoeffer, by devising ingeniously simple and decisive choice assays for challenging RGC axons in chick retinal explant cultures, turned the chemoaffinity hypothesis into a proven theory. His experiments mainly elucidated the principles of FT-interactions, but he also gained conclusive evidence for FF-chemoaffinity, which has received less attention.

In a first set of experiments (Fig. 2A) addressing FT-chemoaffinity he divided the embryonic chick tectum into fifths along its a/p axis [43]. A retinal explant was put onto a coverslip coated with a monolayer of cells dissociated from one fifth of the tectum and a second coverslip, carrying cells from another fifth, was arranged so that the outgrowing axons could freely choose their substrate for extension. In all comparisons, temporal axons preferred cells from the more anterior part of the tectum, while nasals were unresponsive. This elegantly demonstrated the existence of a functional guidance gradient along the a/p axis of the tectum, as suggested by Sperry. Notably, the gradient seemed to be monofunctional, *i.e.*, attractive only or repulsive only, as the axons did not recognize any optimal position inside the gradient, but all went for its anterior end. This is in contrast to the above-mentioned *in vivo* experiments and indicates that some cue was missing in the *in vitro* experiments.

Bonhoeffer went on to use membrane fragments derived from the most anterior and most posterior thirds of the embryonic tectum (Fig. 2A). By establishing the now widely used stripe-assay [44–46], the membrane fragments were arranged as a stripe carpet of alternating lanes of about 100 μm width. Axons emanating from a strip-shaped retinal explant on top of the carpet could freely choose the preferred lane. Temporal axons, in sharp contrast to nasals, consistently decided for the anterior membranes. Thus the axon response was topographically differential, but not topographically appropriate, because the nasals instead of being non-responsive should grow on the posterior membranes, derived from their cognate target, reiterating the notion that guidance was incomplete in the *in vitro* assays. Axonal decision was lost after heat-inactivation of the posterior membrane fragments [47]. This eventually proved the existence of a heat-labile, biochemical axon guidance cue on the tectal membranes, as posited by the chemoaffinity hypothesis. The cue was repellent and distributed as a p > a gradient on the tectum.

Remarkably, in addition to the above-cited evidence supporting FT-chemoaffinity, Bonhoeffer also gained evidence for FF-chemoaffinity. He manufactured Y-shaped growth permissive substrates (Fig. 2B) and put a piece of nasal or temporal retina, respectively, to the ends of the two arms of the Y [48]. Somewhat later, he added a fluorescently labeled query explant to the base of the Y, so that its outgrowing axons could freely choose to join either of the two approaching axon populations. Temporal axons exclusively fasciculated with temporals, whereas nasals did not distinguish. These results provide similarly compelling evidence for the existence of topographically differential FF-interactions among RGC axons, as did the stripe assays for topographically differential FT-interactions.

Conceptually, in addition to proving the validity of the chemoaffinity hypothesis, Bonhoeffer's results showed that the FT-influence on migrating growth cones is separable into components, one of which is a monofunctional repulsive activity, while additional necessary components still seemed to be missing. With regard to the FF-influence, they are consistent with the notion of similar interactions among fibers.

4. The molecular findings completing the picture

The repulsive activity of the chick posterior tectal membranes was found to be ephrin-A5 in a landmark study [49]. At the same time ephrin-A2 and the receptor EphA3 were suggested as further molecular components of the chick's retinotopic guidance machinery [50]. These results put the ephrin/Eph system center stage as topographic mapping cues [51–53]. The Eph receptors, subdivided into the EphA and EphB subclasses form the largest family of receptor tyrosine kinases in the vertebrate genome (mouse: EphA1–A10, except EphA9; EphB1–6, except EphB5). Their binding partners, the ephrins, are correspondingly subdivided into glycosylphosphatidylinositol (GPI)-anchored ephrin-As and transmembrane ephrin-Bs (mouse: ephrin-A1–A5; ephrin-B1–B3). Ephrins and Ephs are thought to interact promiscuously within their subclasses, but only few cross-react (EphA4, EphB2, ephrin-A5, ephrin-B2 and B3). A peculiarity of the ephrin/Eph system is its ability to transduce signals in forward (ephrin > Eph) as well as in reverse (Eph > ephrin) direction. Being GPI-anchored, the ephrin-As need co-receptors for reverse signaling, which were suggested being TrkB in the chick retinotectal [54] and p75NTR in the mouse retinocollicular systems [55]. In the chick, ephrin-A2 and ephrin-A5 form conspicuous p > a gradients in the tectum [49,50] and EphA3 displays a pronounced t > n gradient in the retina [50]. Retinal EphA5-7 mark less obvious t > n gradients and EphA4 is expressed uniformly [56]. Surprisingly, in addition to the tectal expression, prominent n > t gradients of ephrin-A2 [57] and ephrin-A6 were found in the retina [58]. Correspondingly, EphA3 also displays a marked a > p gradient in the tectum [56]. EphA5-7 exhibit less pronounced a > p gradients on the target, while EphA4 is more uniformly expressed [56,59]. Expression patterns in the mouse are indicated in Fig. 2D. Because of the binding promiscuity, it is commonly believed that all co-expressed ephrin-As or EphAs, respectively, can be summed up. Thus the expression patterns in chick and mouse, amount to counter-gradients of ephrin-As and EphAs (Fig. 1B) along the a/p axes of both, the projecting and target fields. Notably, these distributions of ephrin-As and EphAs immediately suggest the possibility of six components of ephrin-A/EphA signaling: (i) FT forward (FTfwd), (ii) FT reverse (FTrev), (iii) FFfwd, (iv) FFrev, (v) *cis*-fwd and (vi) *cis*-rev. In the following, we will

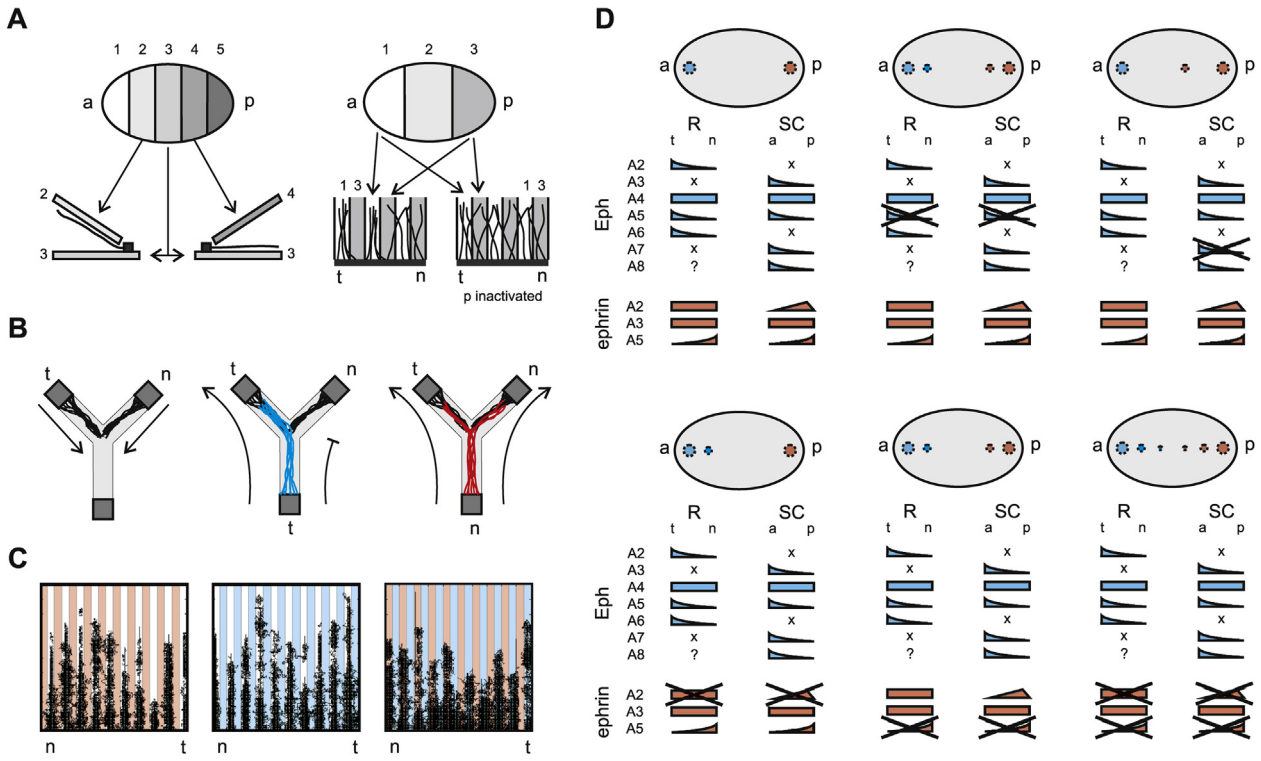

Fig. 2. *In vitro* and genetic findings on the mapping mechanism. (A) Bonhoeffer's *in vitro* experiments demonstrating a monofunctional guidance gradient on the tectal target. Retinal axons consistently prefer to grow on cells derived from more anterior tectal fifths (left). Stripe assays show a repulsive biochemical activity on posterior tectal membranes. Temporal axons, in contrast to nasals, avoid posterior membranes as long as they are not inactivated (right). (B) Bonhoeffer's *in vitro* experiments demonstrating topographically differential FF-interactions. Temporal axons selectively avoid nasals. (C) Simulated single-cue stripe assays corresponding to the *in vitro* experimental results in [60]. Purified ephrin-A (red) and EphA (blue) display repulsive activities. Double-cue stripe assays with ephrin-A/EphA evoke topographically appropriate axon responses (temporals on EphA, nasals on ephrin-A). Shown are the trajectories of simulated axons. (D) Ephrin-A/EphA expression patterns in the mouse retina (R) and superior colliculus (SC) based on [9] and updated from [62,64,82–85]. On top of each column, the collicular phenotypes of the genetic deletions (bold crosses) are shown. Large dots denote normal termination zones, smaller ones indicate eTZs; blue: temporals, red: nasals.

review the findings regarding these six components in retinotopic mapping.

4.1. Fiber–target forward and reverse signaling

Recombinant ephrin-A2 or ephrin-A5, fused to a human antibody Fc fragment to induce dimerization *via* disulfide-bonds required for receptor tyrosine kinase activation, exert repellent activity on chick temporal RGC axons similar to native posterior tectal membranes in stripe assays [60]. At high concentrations, both ephrin-As induce non-differential repulsion of temporal as well as nasal axons (*cf.* Fig. 2C). Lowering the concentration results in a gradual degradation of the decision proceeding from nasal to temporal. This demonstrates a monofunctional action (repulsive only) of the ephrin-As. No concentration is found, at which the axons decide for the ephrin-A, as would be required for a bifunctional cue guiding the terminals by push and pull to a specific destination. When mouse retinal axons were grown on anterior membranes supplemented with increasing proportions of membranes of ephrin-A2 transfected cells, a transition from growth promotion to inhibition was reported relative to the outgrowth on pure anterior membranes [61]. This is, however, not to be mistaken for a bifunctional action of ephrin-A2, because the absolute outgrowth merely declined with increasing ephrin-A2 concentrations. The molecular identification of the topographic guidance cues also enabled their genetic targeting in the mouse. Most ephrin-As and EphAs in mice are expressed in both, the retina and the SC (Fig. 2D) and deleting each one of them unconditionally will affect multiple signaling pathways. Ephrin-A2, however, is graded only in the SC [50] but uniform in the retina [62]. Thus, genetic deletion should primarily affect the differentiality of FTfwd signaling. Most likely due to the redundant expression patterns, no ephrin-A/EphA single knock-out has ever resulted in a complete disruption of the map. Instead, additional ectopic termination zones (eTZs) are typically found, with increasing effects the more paralogs are deleted (Fig. 2D; [62]). Thus, ephrin-A2 deletion [63] yields posterior eTZs of temporal axons (Fig. 2D), consistent with FTfwd signaling mediating repulsion of axons from the posterior tectum also *in vivo*.

The membrane *in vitro* assays failed to identify all components of the FT guidance influence. The presence of ephrin-As on growth cones and EphAs on the target together with the possibility of ephrin-A/EphA bidirectional signaling, however, hinted to reverse signaling as the missing component. The ectodomains of EphA3 [60] or EphA7 [64] fused to human Fc fragments indeed induce distinct repulsion of RGC axons in stripe assays (*cf.* Fig. 2C), proving the existence of robust FTrev signaling. The response in chick retinal axons needs slightly more protein than FTfwd repulsion by the ephrin-As. In the mouse (Fig. 2D), the EphA7 knock-out [64] should mainly effect FTrev signaling, because EphA7 is not expressed in the retina but as a pronounced a > p gradient in the SC. It results in anterior eTZs of nasal axons, suggesting FTrev signaling mediate repulsion of axons from the anterior tectum also *in vivo*, opposing the action of FTfwd signaling. Similarly, loss-of-function of the reverse signaling co-receptor p75NTR causes an anterior shift of the whole labeled nasal subpopulation [55].

The opposing actions of FTfwd and reverse signaling nourished the idea that both might act monofunctionally and combine to provide bifunctional FT-guidance for topographic homing [60]. Double-cue stripe carpets consisting of alternating lanes of EphA3 and ephrin-A2 (*cf.* Fig. 2C) indeed evoked the first topographically

appropriate decisions of chick RGC axons observed with purified guidance cues *in vitro*, with temporals growing on EphA3 and nasals on ephrin-A2. These results clearly demonstrate that a complete topographic guidance system can be composed of two opposing monofunctional cues, like FTfwd and reverse signaling. According to the respective expression patterns (Fig. 2D), the genetic knock-outs of ephrin-A5 [65] and EphA5 [66] should concurrently affect forward and reverse signaling. In fact, both result in combinations of anterior and posterior eTZs, probably arising from reduced reverse and forward signaling, respectively.

4.2. Fiber–fiber forward and reverse signaling

Following up Bonhoeffer's original observations on FF-interactions, RGC fiber collisions were analyzed by time-lapse video-microscopy [67]. When temporal growth cones (high EphA) touched nasal fibers (high ephrin-A) collapse was observed. In other combinations, fibers ignored each other or fasciculated. Removal of axonal ephrin-As by phosphatidylinositol-specific phospholipase C (PIPLC), which cleaves off GPI-anchored proteins, or masking with soluble EphA3 (unpublished observations) attenuated repulsion, but left all other interactions (notably also the fasciculation) unaffected. Therefore, the collision behavior of temporal growth cones seems to be caused by monofunctional ephrin-A/EphA FFfwd signaling. The unresponsiveness of nasal growth cones adds another piece to the evidence for reduced sensitivity of reverse signaling *in vitro*. However, with regard to the *in vivo* situation this conclusion might be misleading. A topographic guidance apparatus relying on forward and reverse signaling would confront nasal axons carrying high concentrations of ephrin-As at the entrance of the tectum with a sharp repulsive boundary of EphA. Hence, nasals might be desensitized per default (and thus also *in vitro*) to overcome the boundary and acquire full sensitivity only inside the target field. That ephrin-A/EphA FFrev signaling might actually play a role *in vivo* is suggested by knock-in experiments, in which a scattered half-population of RGCs was made to express a constant amount of EphA3 (Fig. 1C) not endogenously present in the mouse retina [68]. This leads to map duplication, with the knock-ins mapping in the anterior SC and forcing wild-type axons to map posteriorly. These seminal experiments have actually renewed the interest in FF-interactions. Indirect FF-interaction by a competitive comparison of FT-forward signaling among the growth cones (relative signaling) [69] has been proposed to account for the results, which could, however, more obviously be explained by direct FFrev signaling. In summary, these results notably suggest FF-interactions being mediated by the very same cues as FT-interactions.

4.3. Cis forward and reverse signaling

Evidence for *cis*-fwd signaling comes from stripe assays with reduced concentrations of recombinant ephrin-A2, in which nasals are unresponsive [57]. Removal of axonal ephrin-As by PIPLC renders them sensitive, suggesting that *cis*-interactions had desensitized forward signaling before. Ephrin-As expressed in *cis* with EphAs can in fact silence forward signaling by the ephrin-A molecule binding to a juxtamembrane domain of the receptor, which is not identical with the conventional ligand-binding domain [70]. We would like to call this interaction "molecular *cis*". It has however been doubted whether it actually occurs in native growth cones, because EphAs and ephrin-As seem to be segregated to different membrane micro-domains [71] and start to intermingle only at higher relative ephrin-A concentrations [72]. Due to the multitude of EphAs in the retina such conditions might never be reached in RGC growth cones. Notably, however, one also has to envisage another type of *cis*-interaction, with the binding partners being arranged in *trans* and bending of the intervening membrane. Such "cellular *cis*" interactions will inevitably occur, whenever the highly motile filopodia of a growth cone happen to collide. Cellular *cis* interactions can equally explain the results of the PIPLC sensitization experiments (see Section 5; [60]). The occurrence of *cis*-rev signaling has not systematically been investigated.

In total, there is strong evidence for FTfwd, FTrev, FFfwd and *cis*-fwd interactions in retinotopic mapping. FFrev and *cis*-rev signaling have not yet conclusively been demonstrated, but as the expression patterns and ephrin-A/EphA binding characteristics predict their existence, they should be considered in the absence of any evidence for mechanisms that could selectively suppress these interactions. Conceptually, the molecular evidence identified Bonhoeffer's monofunctional repulsive activity with ephrin-A/EphA FTfwd signaling and indicated that FTrev signaling might be the missing second component of FT-interactions. With regard to FF-interactions, the results are suggestive of the same activities working among fibers.

5. Modeling approaches attempting to reconcile the variegated findings

Since 1975, there have been numerous attempts to understand topographic mapping through computational modeling [73]. Many models yielded valuable insights into components of the mapping machinery, but only few tried to be comprehensive. Along the way, it turned out that it is possible to model partial elements of the machinery based on highly divergent assumptions. Even complex features like map segregation in EphA3 knock-in mice, for example, can be reproduced assuming a combination of weak, merely permissive target cues, non-selective competition and sophisticated forms of Hebbian activity-dependent plasticity [74]. Primary topographic mapping, however, is activity-independent [27]. Thus, the challenge is to find a model, which starts out from experimentally validated mechanisms, but still yields the observed systemic features.

A seminal notion for a theoretical understanding of topographic mapping has most lucidly been formulated by Gierer [75]. According to this concept, axonal guidance to the target is conceived as a potential minimization process with the growth cones moving down the potential gradient brought about by the graded distribution of the guidance cues. Gierer pointed out that the potential trough could be generated either by one bifunctional concentration gradient, if the response to the cue is highly non-linear, *e.g.*, attractive before and repulsive behind the target. Alternatively, the potential minimum could be assembled by two monofunctional gradients, *e.g.*, by counter-gradients of two repulsive influences.

The above-mentioned evidence reveals that actually two potential troughs are required for a comprehensive model (Fig. 3A). The first minimum is needed to describe FT-interactions and, thus, to account, *e.g.*, for the time-courses of flexible map adjustments, single axon mapping or the "follow" result of tectal graft experiments as well as the stripe assay results. A second influence on growth cone guidance is needed to account for the mapping flexibility experiments (*e.g.*, expansion, compression, mismatch), which suggest additional FF-interactions. These interactions need to be topographically selective, *i.e.*, they need to form a potential minimum, when the terminal has arrived at its topographically appropriate neighbors (Fig. 3A), to explain, *e.g.*, the "ignore" result of tectal graft experiments or the polarity reversal experiment. The latter results, in addition, indicate that the topographic selectivity of the FF-interactions must be autonomous from the target by demonstrating that fibers can self-sort even against the target cues.

Using these requirements as a benchmark, five comprehensive models have been proposed up to now, three before the full elucidation of ephrin-A/EphA mechanisms [76–78] and two

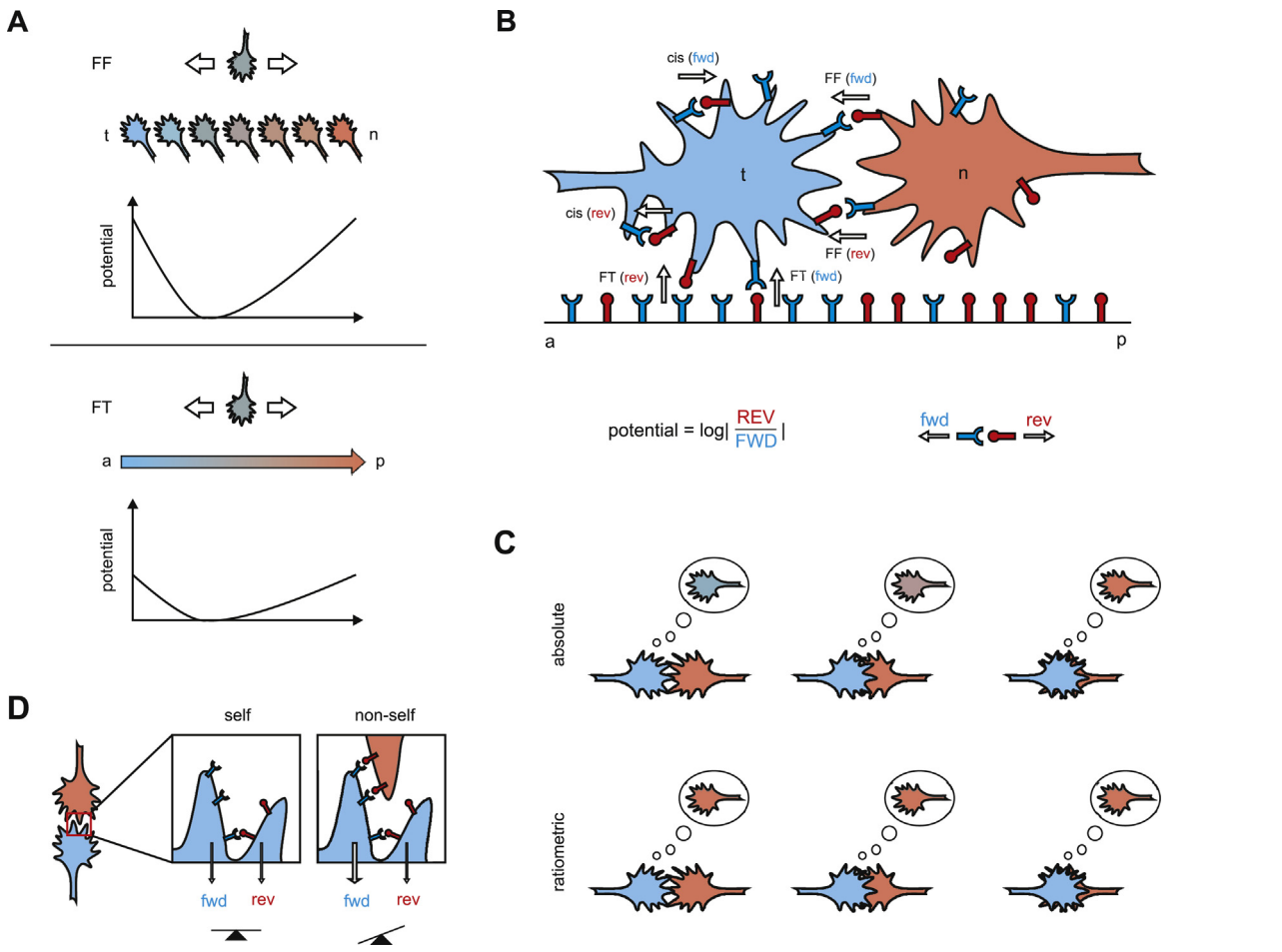

Fig. 3. Computational modeling of FF- and FT-chemoaffinity. (A) Minimal requirements for a comprehensive model of robust mapping. Topographically selective FT- and FF-interactions, each giving rise to an independent (separating line) guidance potential trough. (B) The model introduced in [60] implements these requirements by exclusively relying on all six dimensions of ephrin-A/EphA signaling (FTfwd, FTrev, FFfwd, FFrev, *cis*-fwd, *cis*-rev). The guidance potential is calculated by adding all instantaneous forward and reverse signals impinging on the growth cone, respectively, and balancing the sums. The target is reached when the guidance potential is zero. (C) Distance dependence of absolute signals in FF-interactions is eliminated by ratiometric sensing requiring two signaling channels. (D) Self/non-self discrimination can be achieved by utilizing reverse signaling. Balancing indicates self-signals, unbalancing non-self signals.

thereafter [60,79]. The seminal marker induction model [78,80] suggests that the virgin target just carry weak orienting signals. The invading fiber terminals induce the actual cues (*e.g.*, ephrin-A expression [80]) according to their own marker endowment. Inductive strength is dynamically adjusted in proportion to the degree of marker matching between fiber and target points and terminals can sprout or prune branches depending of their inductive success. The target cells communicate to trade off their marker expression. Thus, terminals from neighboring origins cooperate, while terminals from non-neighboring origins interfere through the target. Due to the fiber-dependence of target specification this indirect FF-interaction is quasi-autonomous, as required. The target is proposed to have a memory of the induction. Albeit very powerful in reproducing the experimental observations, evidence for the induction mechanism has remained scarce. The multiple constraints model [76] is similarly powerful and of exemplary lucidity regarding the underlying concepts. Being published before the identification of the ephrins, it draws on attractive interactions and, unfortunately, has not been updated. The Cowan model in its latest implementation [77] depicts the mapping process in terms of the dynamics of synaptic weight matrices described by differential equations. Although theoretically comprehensive, it has remained difficult to relate to experimentally approved mechanisms. After the corresponding signaling pathways had been elucidated, a first model has been proposed that relies on ephrin-A/EphA based FTfwd, FTrev, FFfwd and FFrev signaling [79]. Mapping is assumed to occur through interstitial branching from pre-positioned main axons. This mechanism strongly limits the dynamics and impedes the predictive power of the model. Thus, it fails, *e.g.*, to appropriately reproduce the map segregation observed in EphA3 knock-in animals.

We have recently proposed a comprehensive model (Fig. 3B), which builds on growth cone migration [60]. The model builds on the concept that all major a/p guidance interactions might be due to ephrin-A/EphA signaling and fully relies on all of its six dimensions: FTfwd and FTrev to implement FT-chemoaffinity, FFfwd and FFrev to implement FF-chemoaffinity as well as *cis*-fwd and *cis*-rev signaling in the form of "cellular *cis*" interactions (see Section 4.3). All pathways are equally weighted except FF *trans* signaling, which is assumed to gain importance during development to mirror the growth of terminals from growth cones to terminal arborizations. The guidance potential is calculated by summing up all instantaneous forward and reverse signals impinging on a migrating growth cone independently and eventually balancing the two sums. The target is reached, *i.e.*, the potential is minimized, when both are equal. The model in addition to generating a normal topographic map (Fig. 1B) successfully reproduces the evidence from stripe assays (Fig. 2C), including the *cis*-signaling assays, single axon mapping, expansion (with correct developmental time-course and on empty as well as preoccupied targets), compression, mismatch,

polarity reversal as well as map segregation in hetero- and homozygous EphA3 knock-in animals (Fig. 1D). The model can also simulate the phenotype of Math5 deletion mutant mice (Fig. 1D), which has recently revived the interest in competition models [81]. In these animals a severely reduced population of scattered RGCs (5–10%) generates a map that fails to cover the SC up to its posterior end. In the model, this phenotype is produced without non-selective competition, because remote from their targets, terminals prefer to stick to topographically similar terminals (if available) to reduce their guidance potential. Inherent limitations of terminal arbor size prevents the small population of interacting terminals from covering the whole target in contrast to the expansion experiments, where the remaining population is much larger. Lacking implementations of activity the model fails in compacting eTZs.

6. Outlook: Ephrin/Eph signaling–perfectly adapted to support fiber–fiber interactions

All comprehensive models agree on the notion that FF-interactions eventually supersede FT-influences. What might be the advantages of FF-chemoaffinity? Embryologically, the retina is derived from the diencephalon, while the tectum is of mesencephalic origin. Thus, both regions develop largely independently. Most likely it would be very difficult and non-robust to generate numerically matching gradients of guidance molecules on both fields, as required by absolute FT-chemoaffinity. If the cues, instead of being tethered to the target are also carried by the motile terminals, robustness can be achieved. The FF-chemoaffinity machinery amounts to the fiber terminals bringing to the target the apparatus to achieve a mapping that suits their needs.

To enable topographic mapping through FF-chemoaffinity, the terminals must carry signals encoding their topographic identity and the corresponding receptors. These requirements pose two major problems. First (Fig. 3C), the signal that one terminal sees when encountering another will not only depend on topographic identity but also on the distance between the two terminals, potentially confounding the mapping. This ambiguity could be easily resolved by utilizing ratiometric measurements, necessitating two signaling channels instead of one. Calculating the ratio of the two channels would eliminate the distance-dependence and render the signal an unambiguous indication of topographic identity. Second (Fig. 3D), the concurrent presence of signals and their cognate receptors on the same growth cone will inevitably entail stochastic *cis*-signaling due to membrane ruffling or collisions of filopodia. Therefore, a self/non-self discrimination problem arises. A parsimonious solution to this problem would be to employ reverse in addition to forward signaling. Balanced signals (or signals at any preset ratio, if binding constants for both pathway directions are not equal) will then indicate self-signals. Unbalances, in contrast, will imply non-self signals with the unique exception of encountering identical terminals. This, however, is irrelevant for further guidance as it signifies target arrival. Hence, combining forward and reverse signaling can solve the problem of self/non-self discrimination, but at the same time it also obviates the need for an independent second signaling channel for ratiometric sensing, because the two pathway directions might be employed as the two channels. Thus, the ephrin/Eph system, providing for these mechanisms, seems perfectly adapted for a FF-chemoaffinity based machinery for robust topographic mapping. Wiring economy has been suggested being a major advantage of topographic projections. Considering the increase in wiring complexity from lower vertebrates to our brain casts doubts on this argument. Maybe, the evolutionary advantages of having a powerful brain outperform the disadvantage of having to invest more energy in the wiring. Conceivably, a better argument might be the regulatory simplicity by which mass wiring can be achieved based on the amazing mechanism of topographic mapping reviewed here.

Funding

This work was supported by the German Research Foundation (DFG; grant BA1034/14-3 to F.W. and M.B.; KSOP grant GSC21 to F.F. and C.G.).

Acknowledgments

The authors would like to thank Markus Weschenfelder for critically reading the manuscript and Friedrich Bonhoeffer for ongoing inspiring input.

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